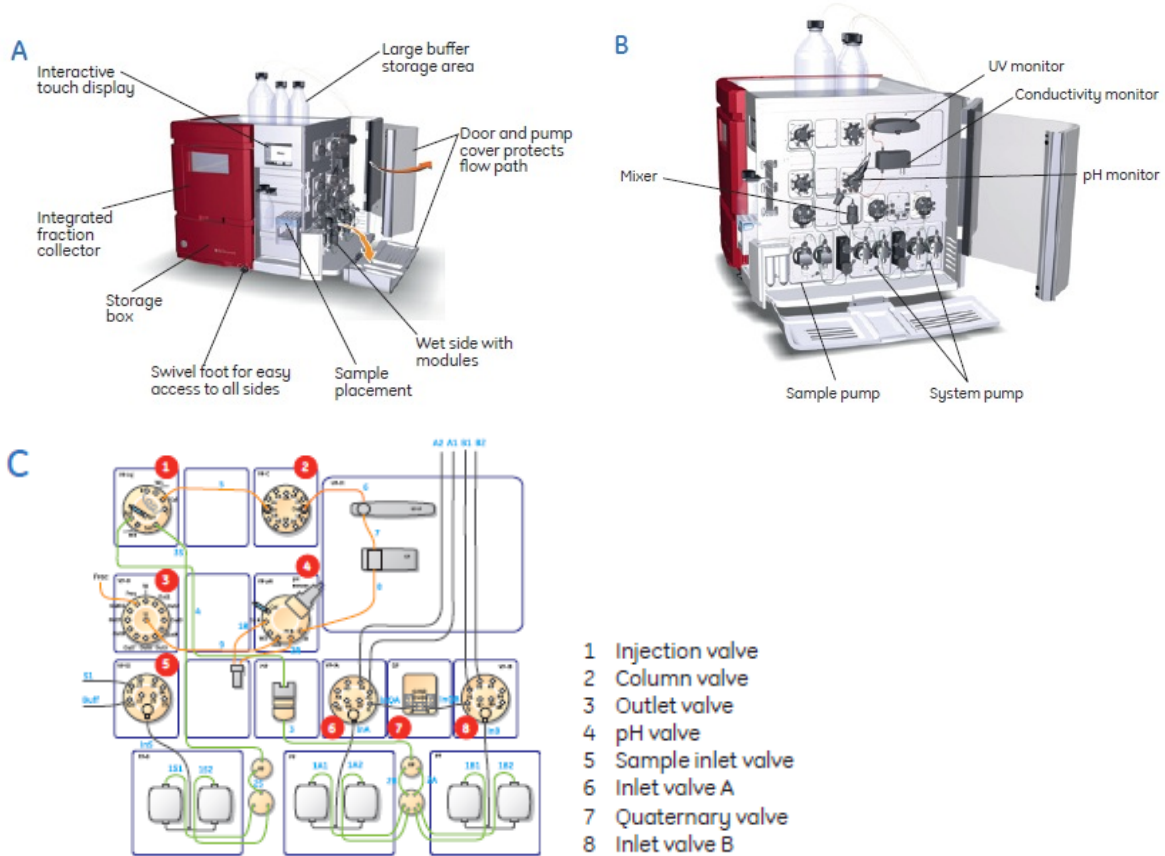


FPLC Protocol - (Akta Avant)

General AKTA setup



Start up protocols:

1. First ensure that the AKTA lines have been stored in 20% ethanol (any time the machine will not be in use it should be flushed and stored in 20% ethanol).
2. The column is also stored in 20% ethanol at 4°C
3. The column should then have the appropriate valves inserted (see below) to be placed it on the AKTA.



4. Columns are always placed in position two of the Akta Column valve



5. Move all lines that will be used (from pump A, B and sample pumps) to DI water. Any lines not in use should remain in the 20% ethanol
6. Purge all lines before running a method (see purging protocol)
7. System and column should be flushed with DI water (at least 10 ml for all lines and 3-5X column volumes (CV) for the column (See start up protocol: Glenna/HIC sysprep WWB for example)
8. All lines and columns should then be primed with the appropriate buffer (note: if you plan to use the sample pump to inject your sample you will need to flush the sample line AND the buffer line).

Starting a method:

1. Before starting a method
 - a. Check method to ensure you have changed the column volume and flow rates so they match the attached column
 - b. Double check that the all lines are in the correct buffer and are either weighted down or taped to the bottom
 - c. Make sure that the 96 well plates have been placed in the fraction collector
 - d. prepare sample and place in line
2. Start method

Cleaning method: (see HIC sysprep H2O EtOH shutdown file for example)

1. After method, all lines should be moved to a DI water bottle and flushed with at least 10 ml of water (for column at least 5 CV if it has not been flushed yet in the purification protocol)
2. All lines and the column should then be flushed in EtOH (10 ml for each line, or 3 CV for the column)

3. Column can then be removed from AKTA, and the storage stoppers replaced. Store column in fridge.
4. Reconnect lines where column is removed and leave all lines in 20% ethanol container.

Purging protocol (lines must be purged of air before use):

1. In System Control window: in file menu select Manual
2. Go to Execute manual instructions, under pumps and pressures select pump wash
3. Select which lines to purge and click "Insert" button to add to Instructions execution list
4. Hit execute and pumps will start
5. Take syringe with tubing and connector and insert it into the active pump's valve (either of the valves will do) See picture below.
6. Open valve and collect liquid until the line has been purged of all air
7. Close valve and stop pump by hitting stop button in System control main window
8. Repeat procedure for other pumps until all have been purged.

